Stem Cell Research 17 (2016) 158–160

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Generation of human iPS cell line SKiPSc1 from healthy Human Neonatal Foreskin Fibroblast cells

Abdullah Alawad [a,1], Othman Alhazzaa [a,1], Saleh Altuwaijri [b,c], Mohammad Alkhrayef [a], Faisal Alagrafi [a], Ziyad Alhamdan [a], Abdullah Alenazi [a], Sultan Alharbi [a], Mohamed Hammad [a,c,*]

[a] National Center for Stem Cell Technology (NCSCT), Life Sciences and Environment Research Institute (LSERI), King Abdulaziz City for Science and Technology (KACST), Riyadh 11442, Saudi Arabia
[b] Department of Surgery, University of Michigan, Ann Arbor, MI 48109, USA
[c] SAAD Research & Development Center, Clinical Research Lab.& Radiation Oncology, SAAD Specialist Hospital. P.O. Box 30353, Al Khobar 31952, Saudi Arabia

ARTICLE INFO

Article history:
Received 7 June 2016
Accepted 15 June 2016
Available online 25 June 2016

ABSTRACT

The SKiPSc1 induced pluripotent stem (iPS) cell line was generated from Human Neonatal Foreskin Fibroblasts (HNFFs) obtained from a healthy donor infant that were reprogrammed using non-integrating Sendai viral vectors expressing Oct3/4, Sox2, c-Myc, and Klf4.

Resource table

Name of Stem Cell line	SKiPSc1
Institution	KACST
Person who created resource	Othman Alhazzaa
Contact person and email	Abdullah Alawad, alawad@kacst.edu.sa
Date archived/stock date	January 1, 2016
Origin	Human Neonatal Foreskin Fibroblasts (HNFFs)
Type of resource	Biological reagent: iPS cell line derived from HNFFs
Sub-type	cell line
Key transcription factors	Oct4, Sox2, cMyc, Klf4
Authentication	Identity and purity of cell line confirmed (Fig. 1)
Link to related literature	N/A
Information in public databases	N/A
Ethics	Patient informed consent obtained/Ethics Review Board-competent authority approval obtained

Resource details

To generate the Saudi KACST induced Pluripotent Stem Cell line No 1 (SKiPSc1) cell line, four reprogramming Yamanaka factors (Oct3/4, Sox2, c-Myc, and Klf4) (Takahashi et al., 2007; Yu et al., 2007) were delivered to HNFFs by Sendai viral infection (Life Technologies, Invitrogen) (Ban et al., 2011). SKiPSc1 colonies exhibited a characteristic round shape morphology with small, tightly packed cells with a high nucleus/cytoplasm ratio and prominent nucleoli (Fig. 1A). The Sendai virus is non-integrating and the absence of exogenous reprogramming factors and the SeV vector in the cells was confirmed by PCR analysis (Fig. 1B). Pluripotency was verified by endogenous gene expression of pluripotent stem cell markers Oct4, Sox2, Nanog, Klf4 and c-MYc by PCR (Fig. 1B). The colonies were also positive for alkaline phosphatase activity (Fig. 1C). Furthermore, pluripotent surface marker expression of Tra-1-60, Oct4 and SOX2 was confirmed by immunostaining (Fig. 1D). Finally, iPSCs single cells were able to generate embryoid bodies when cultured under certain conditions (Fig. 1E).

1. Materials and methods

1.1. Neonatal foreskin samples collection and processing

Neonatal foreskin samples were obtained after routine circumcision of newborn males. Informed consent was obtained from the child's father. The foreskin tissue was removed according to Good Clinical Practice (GCP) and conventional aseptic techniques. Collected samples were transferred to 15 ml plastic test tubes (Thermo Fisher Scientific, Inc) with 5 ml Dulbecco's Modified Eagle's Medium (DMEM, LifeTechnologies) supplemented with 10% fetal bovine serum (FBS, LifeTechnologies) and penicillin (100 U/ml)-streptomycin (100 μg/

* *Corresponding author at: National Center for Stem Cell Technology (NCSCT), Life Sciences and Environment Research Institute (LSERI), King Abdulaziz City for Science and Technology (KACST), Riyadh 11442, Saudi Arabia.
E-mail address: drhammad77@gmail.com (M. Hammad).
[1] Denotes equal contribution

http://dx.doi.org/10.1016/j.scr.2016.06.006

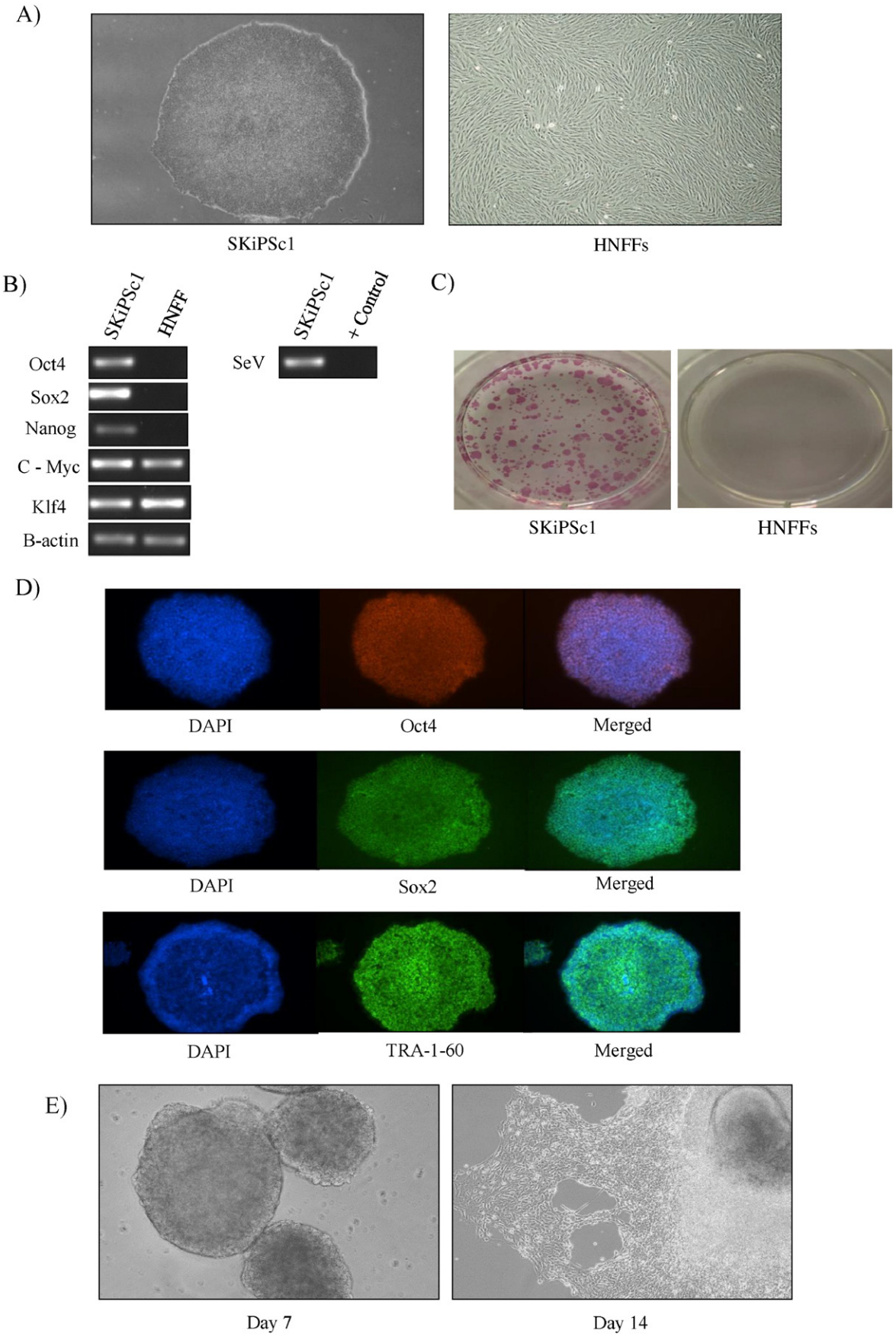

Fig. 1. A) Phase contrast image of HNF cells and SKiPSc1. **B)** PCR analysis of gene expression of pluripotency markers, and Sendai virus vector (SeV). **C)** Alkalin staining of HNF and SKiPSc1. **D)** Detection of pluripotency markers in SKiPSc1 by Immunofluorescence. **E)** Embryoid bodies (EBs) at day 7, and emerging cells from attached EBsat day 14.

ml). Three pieces of explants from each minced foreskin specimen were placed in separate wells of a six-well plate (duplicated) and allowed to adhere to the plastic surface for approximately 5 min at room temperature. DMEM, supplemented with 10% FBS, 100 U/ml of penicillin, and 100 μg/ml of streptomycin in 1 ml aliquots was added to each well. The explant fibroblast cultures were maintained at 37 °C in 5% CO2 and 95% room air. The explant cultures were evaluated under phase contrast microscopy.

Human dermal fibroblasts (passages 3–5) from neonatal foreskin explants were grown in T-75 flasks (Thermo Fisher Scientific, Inc) and subcultured in 6- or 24-well plates (Thermo Fisher Scientific, Inc) using DMEM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml). Fibroblast cultures were grown and incubated at 37 °C in 5% CO2 and 95% room air.

1.2. iPS cell reprogramming

The HNFFs were expanded and reprogrammed. Briefly, HNFFs were expanded in DMEM medium (LifeTechnologies) containing 10% FBS (Life Technologies) and GlutaMAX (Life Technologies). Cells were transduced with Sendai viral vectors expressing human Oct3/4, Sox2, Klf4, and c-Myc. (Life Technologies) (MOI according to manufacturer's instructions). Transduced cells were plated on one well of 6-well plate. SKiPSc1 was cultured in DMEM/F12 with GlutaMAX (Life Technologies), 10% FBS (Life Technologies. The medium was replenished every two days for one week. At day 7, cells were detached using 0.05% Trypsin-EDTA (LifeTechnologies) and plated on 100 mm non-treated dish coated with Vitronectn containing DMEM/F12. The following day, medium was changed to E8 medium (Life Technologies), the medium was replenished every two days. The Cells were maintained in these conditions until uniform colonies were generated and iPS cell colonies were mechanically isolated for expansion in feeder free conditions on Vitronectin in the presence of E8 medium. iPSC lines were routinely split using 0.5 mM EDTA and thawed in the presence of kinase inhibitor (Y-27,632, Sigma) during 24 h after thawing.

1.3. Alkaline phosphatase (AP) staining

AP staining was performed on one well of 6-well plate using the alkaline phosphatase staining Kit (STEMGENT, USA) according to the manufacturer's directions. Colonies were identified based on the following morphological criteria: well defined-border, three-dimensionality, and tightly packed cells.

1.4. PCR

RNA was isolated from the SKiPSc1 cells using the RNeasy micro kit (Qiagen) according to the manufacturer's instructions. Reverse transcription was performed on total RNA (500 ng) using random hexamers with Superscript III Reverse Transcriptase (Life Technologies). The cDNA was used as a template for PCR amplification of the viral backbone and reprogramming genes (SeV, Klf4, Oct3/4, Sox2, Nanog and c-Myc). Primer sequences were provided by the company (Life Technologies). Primer sequences (5` to 3`) were as follows: OCT4_F: CCTCACTTCACT GCACTTGTA; OCT4_R: CAGGTTTTCTTTCCCTAGCT; SOX2_F: ATGTCCCA GCACTACCAGAG; SOX2_R: GCACCCCTCCCATTTCCC; NANOG_F: TTTG TGGGCCTGAAGAAAACT; NANOG_R: AGGGCTGTCCTGAATAAGCAG; KLF4_F: GGTCGGACCACCTCGCCTTACAC; KLF4_R: CTCAGTTGGGAACT TGACCA; c-Myc_F: CTGAAGAGGACTTGTTGCGGAAAC; c-Myc_R: TCTC AAGACTCAGCCAAGGTTGTG; B-Actin_F: AAACTGGAACGGTGAAGGTG; B-Actin _R: AGAGAAGTGGGGTGGCTTTT PCR products were separated on a 2.0% agarose gel and visualized with ethidium bromide.

1.5. Immunostaining

For immunostaining of iPSC, cells were fixed for 20 min at room temperature in 4% paraformaldehyde solution in PBS; they were then washed several times in PBS, and blocked for 30 min in PBS with 5% fetal bovine serum. Cells were then stained with primary antibodies overnight at 4 °C, rinsed with PBS, and incubated with secondary antibodies for 1 h at RT. Primary antibodies used for characterization of iPSC and iPSC-derived cells were TRA-1-60 (SANTA CRUZ BIOTECHNOLOGY, INC, # sc-21705), OCT4 (SANTA CRUZ BIOTECHNOLOGY, INC, #sc-5279,) and SOX2 (SANTA CRUZ BIOTECHNOLOGY, INC, #sc-17,320). Texas Red-conjugated goat-anti-mouse IgG (SANTA CRUZ BIOTECHNOLOGY, INC, #sc-2781,), FITC-conjugated donkey-anti-goat IgG (SANTA CRUZ BIOTECHNOLOGY, INC, #sc-2024), and FITC-conjugated goat-anti-mouse IgM (SANTA CRUZ BIOTECHNOLOGY INC, # sc-2082) were used as secondary antibodies. DAPI was used for counterstaining. Stained cells were analyzed by using IN Cell Analyzer 2000 Imaging System (GE Healthcare Life Sciences).

1.6. Embryoid body (EB) formation

iPSC colonies were passaged as small aggregates using 0.05% Trypsin-EDTA and suspended in lEB medium: Knockout™ DMEM/F12 (Life Technologies) with GlutaMAX (Life Technologies), 10% KnockOut Serum Replacement (Life Technologies), 0.1 mM 2-mercaptoethanol (sigma), 1 × Non-Essential Amino Acids (Life Technologies) The cell aggregates were cultured in suspension in untreated Petri dishes to generate embryoid bodies. After 7 days, embryoid bodies (EBs) ± were plated onto gelatin-coated 6-well plates and incubated for another 14 days for spontaneous differentiation.

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